Experiment 2

FISH, FACS AND CELL COUNTS

GROUP 2

Experiment two

Outline

- Aim
- Introduction
- Method
- Results
- Discussion
- Our own experiments

Experiment two

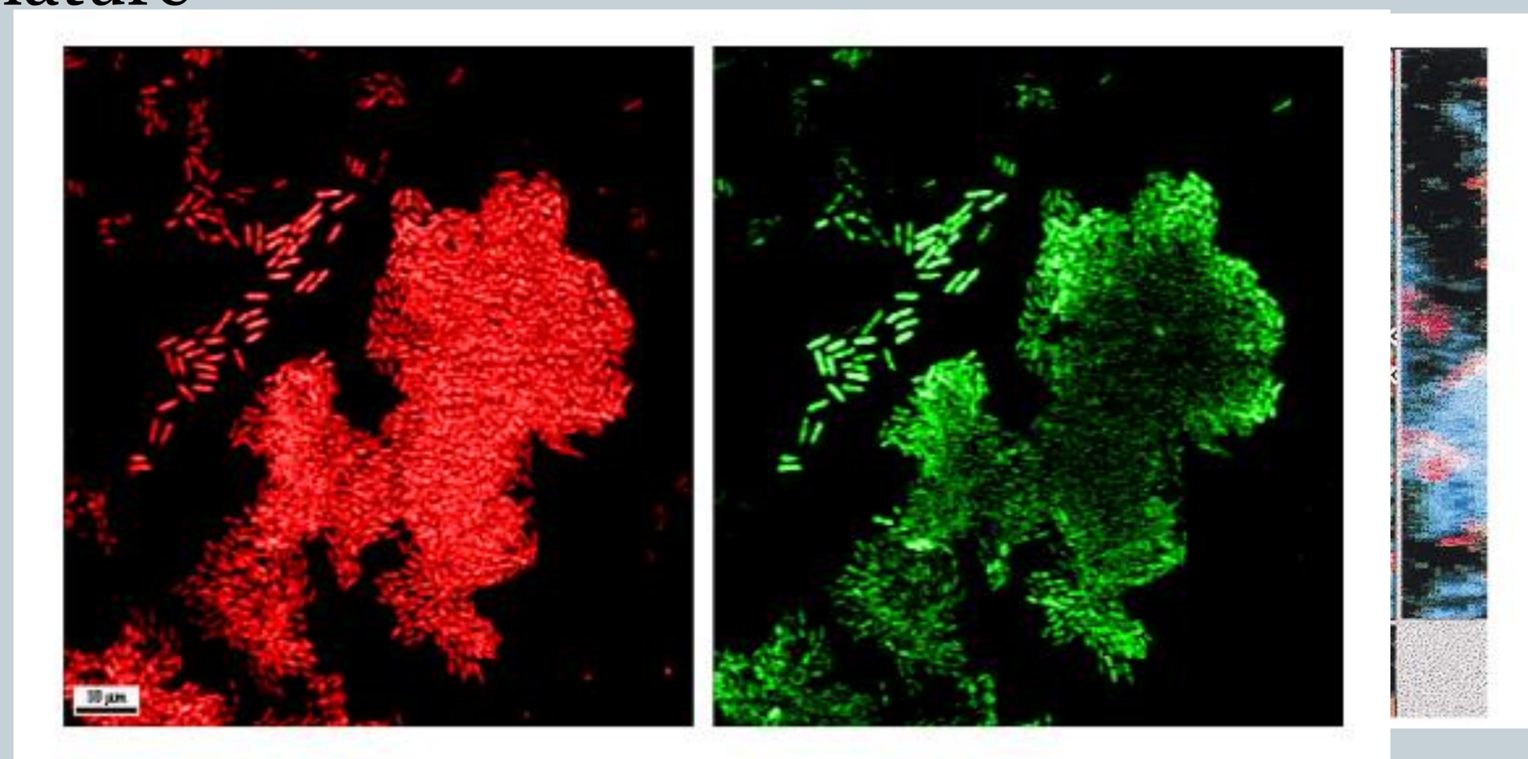
Aim

To examine the multispecies biofilm of *P. aeruginosa* and *Acinetobacter*

Learn new biofilm techniques

Introduction

- Most biofilm found is believed to be multispecies in nature

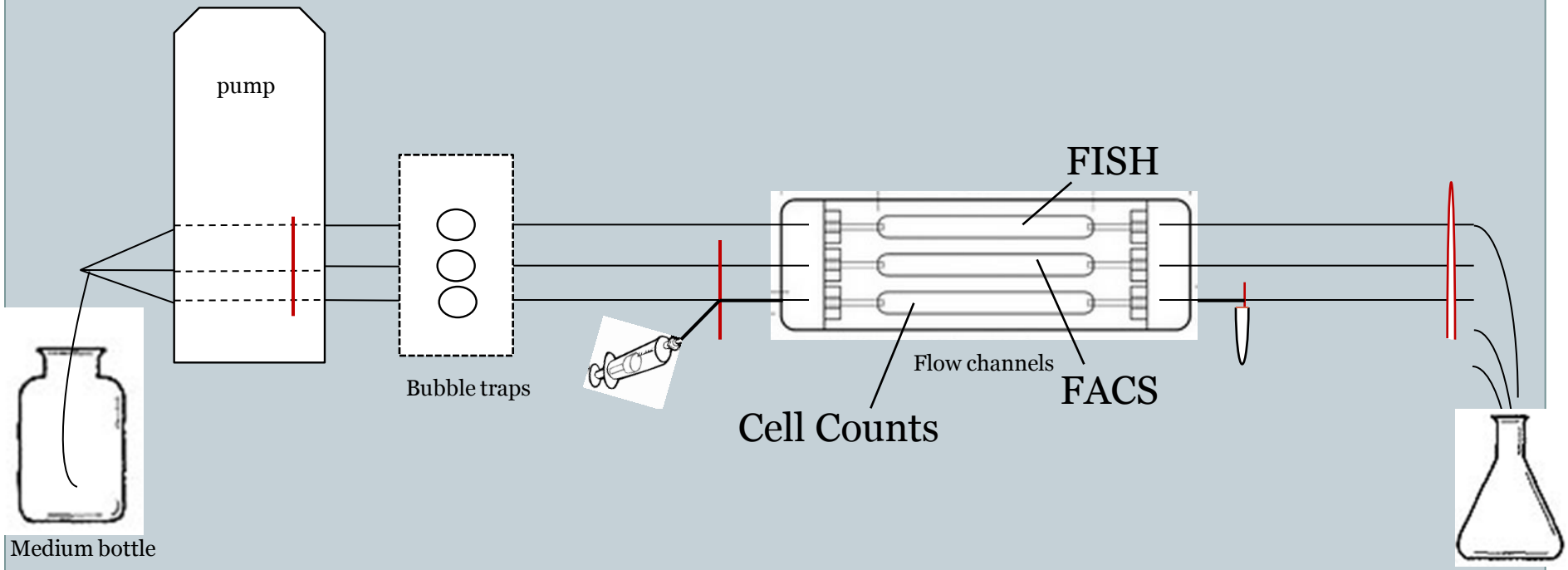


Stellar, Appl Environ Microbiol. 1999 September; 65(9): 4108–4117.

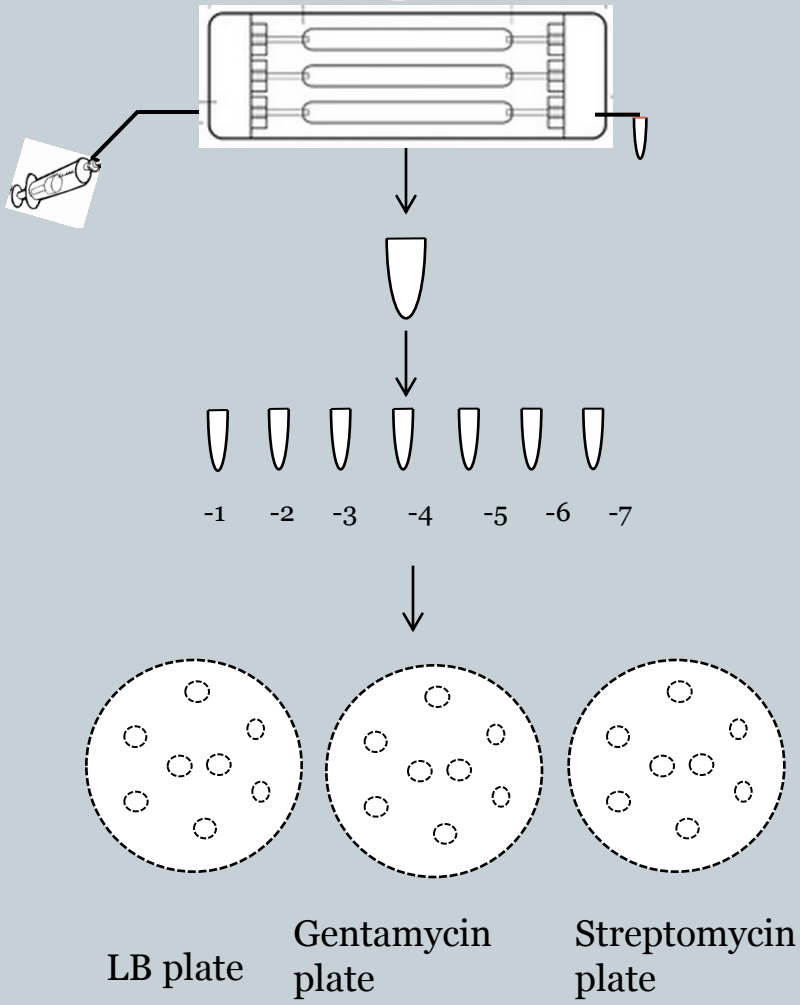
Moller, et al. Appl Environ Microbiol. 1998, 64(2):721

Method

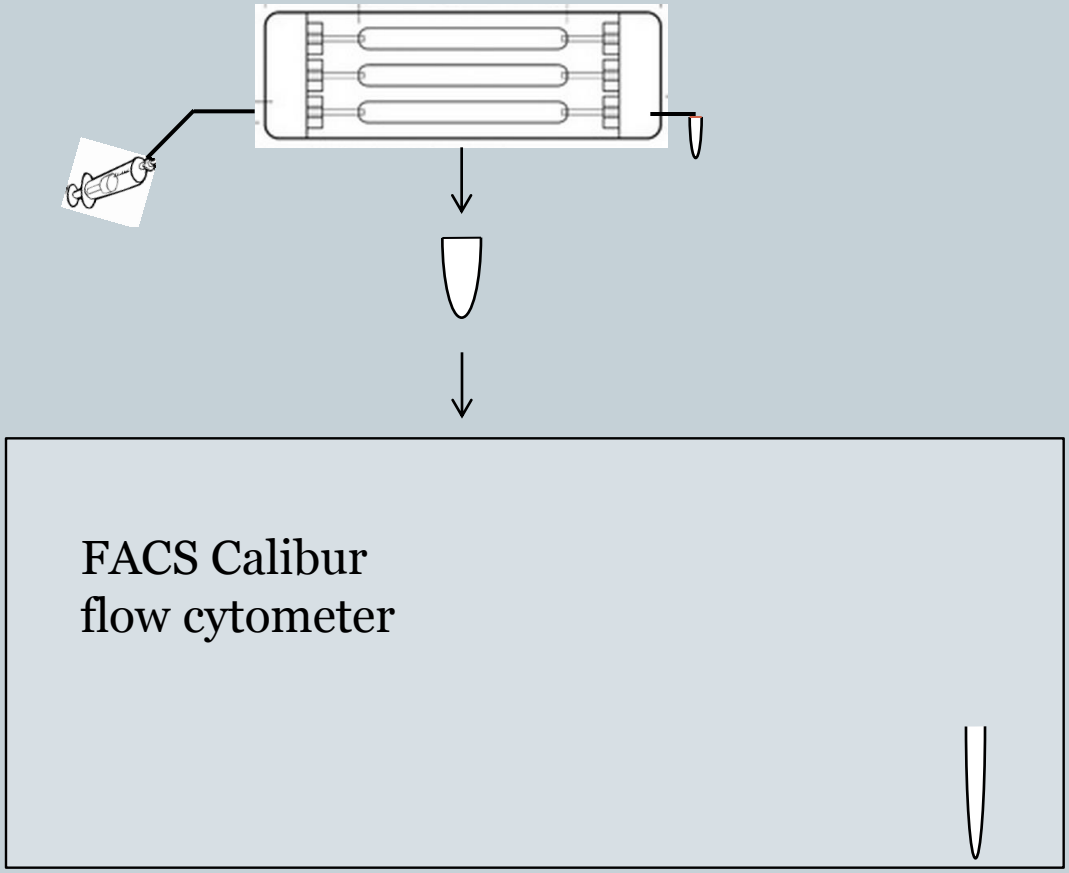
P. aeruginosa ⟶ Dilute 500 times
Acinetobacter ⟶ Dilute 5 times
} Mixture(1:1) ⟶ Inoculate



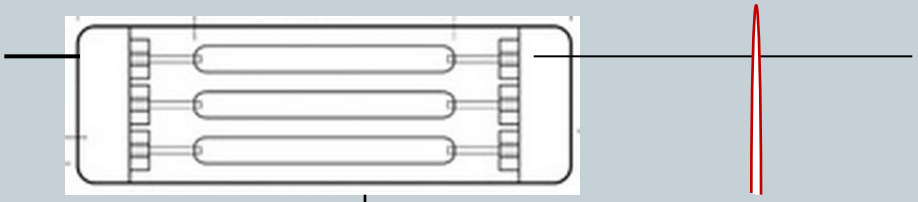
Methods-Cell Counts



Methods-FACS



Method-FISH



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Fixation

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Embedding

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Removing embedded biofilms from the flow chambers

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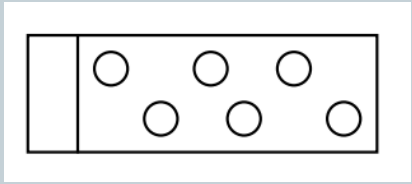
Hybridization of embedded biofilms
Washing procedure



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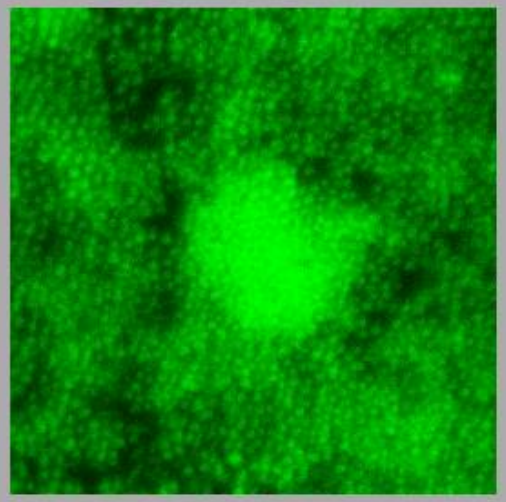
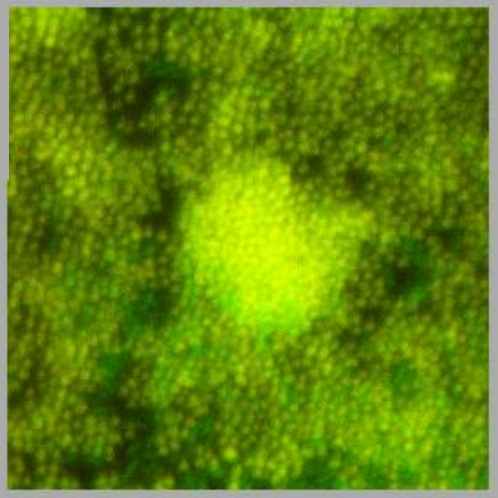
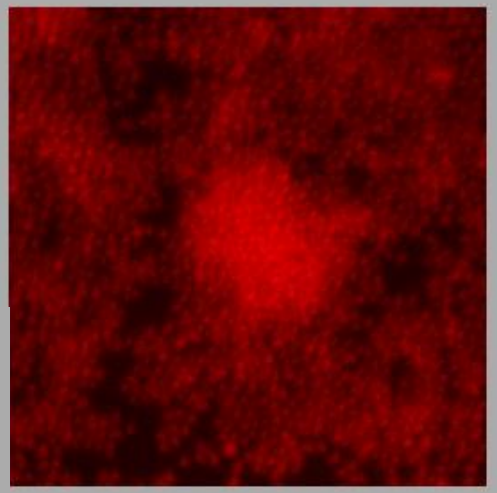
Microscope

Results

- FISH

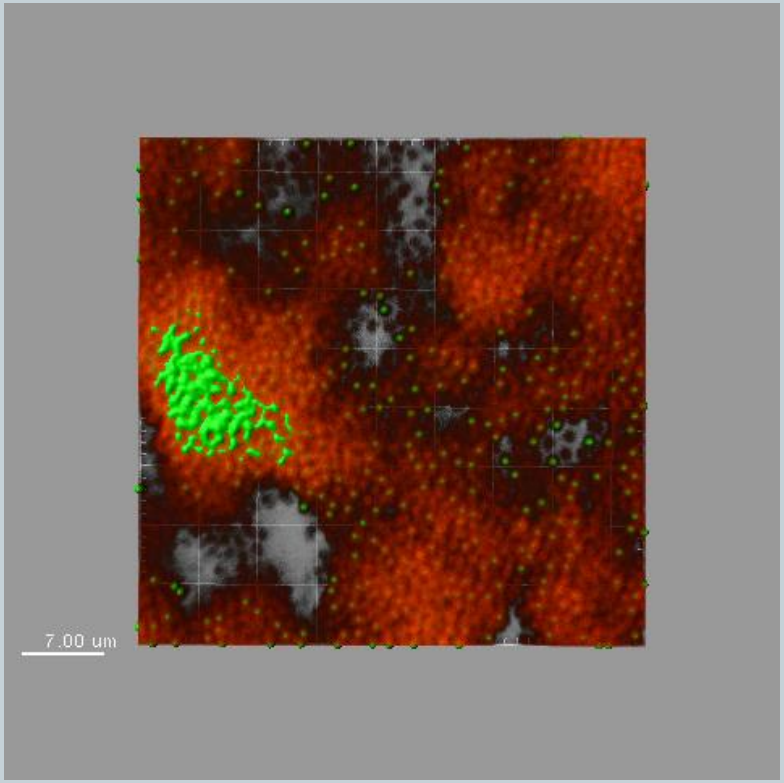


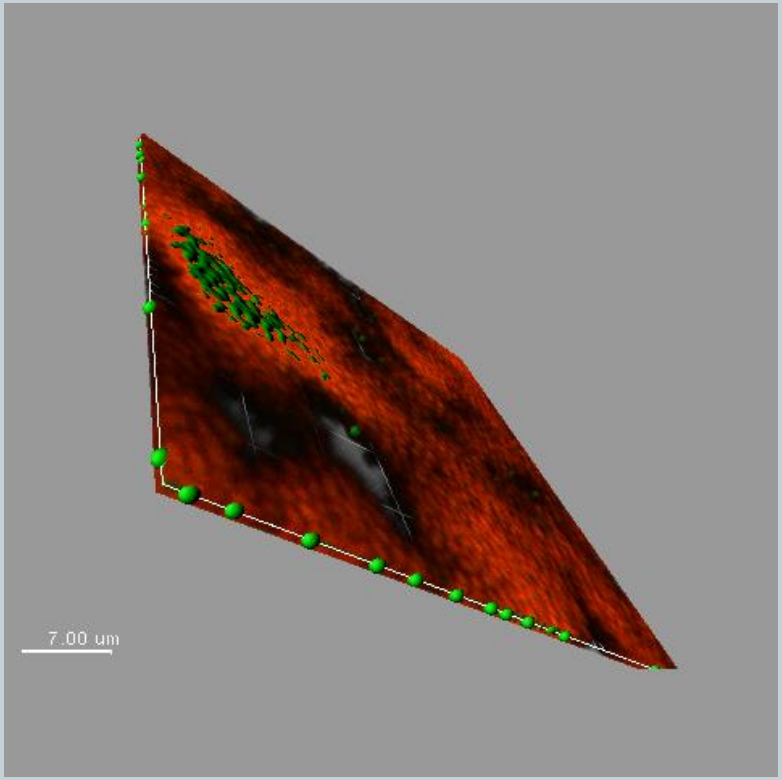




Results

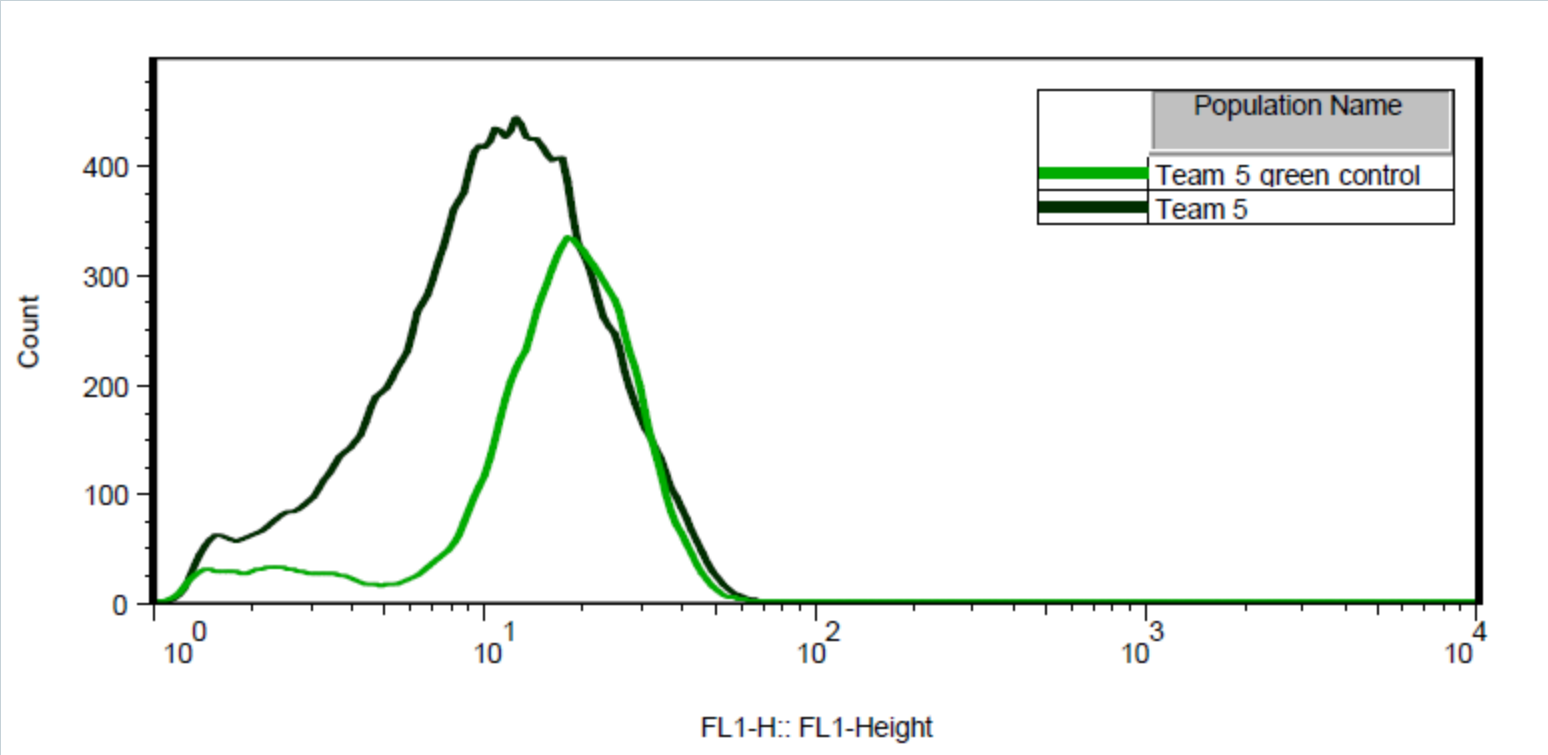
- FISH





Results

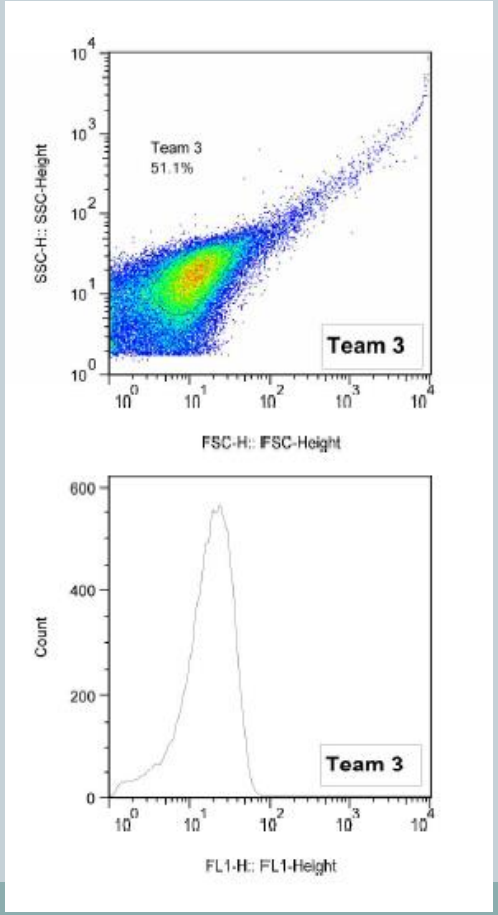
- FACS



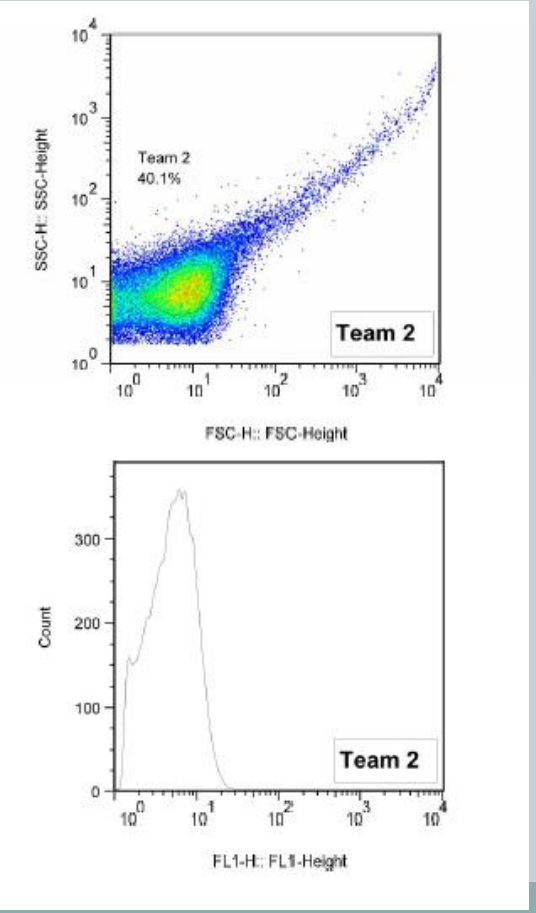
Results

- FACS

P. aeruginosa

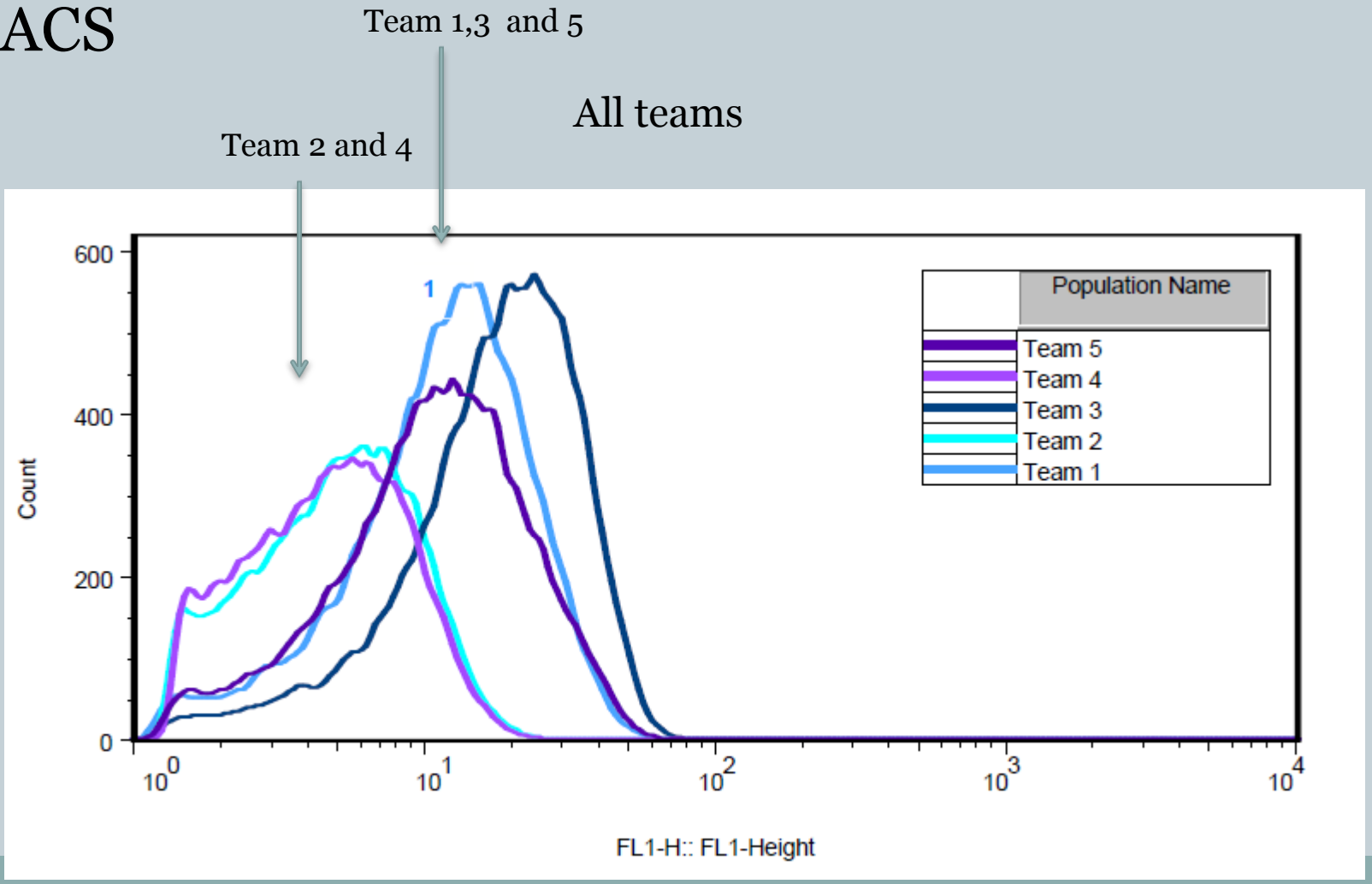


Acinetobacter



Results

- FACS



Results

- Cell counts

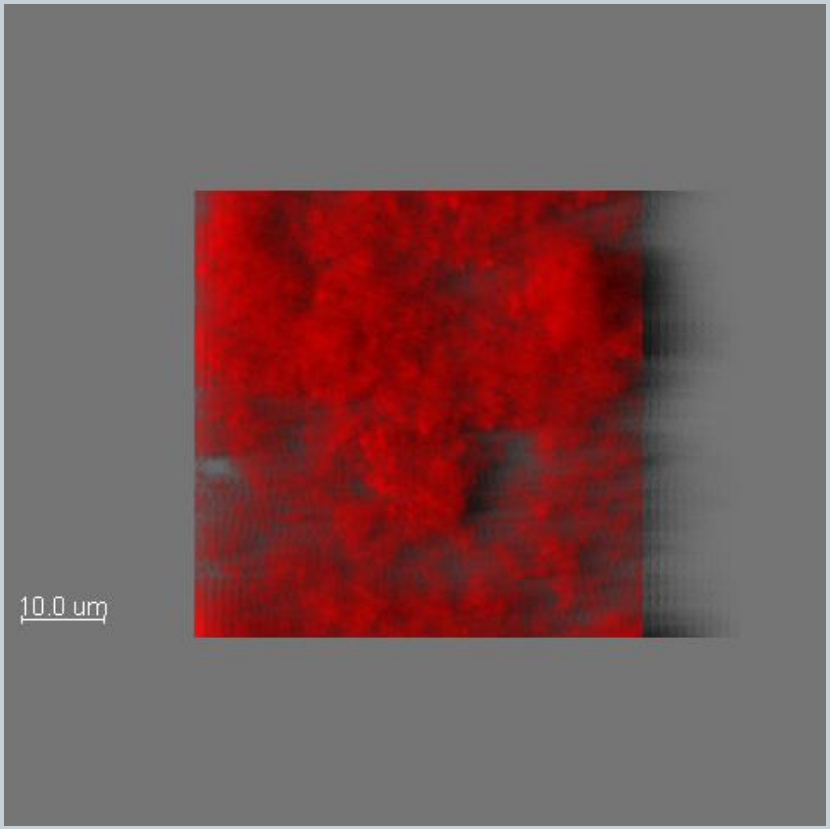
Determination biofilm population (CFU/ml)			
	LB	Gentamycin	Streptomycin
	Both	*P. aeruginosa*	*Acinetobacter*
Group 1	5,2*10^8	-	-
Group 2	1*10^8	2,68*10^8	-
Group 3	1,3*10^7	1,5*10^7	-
Group 4	8,4*10^7	8*10^7	-
Group 5	2,2*10^9	2,3*10^9	-

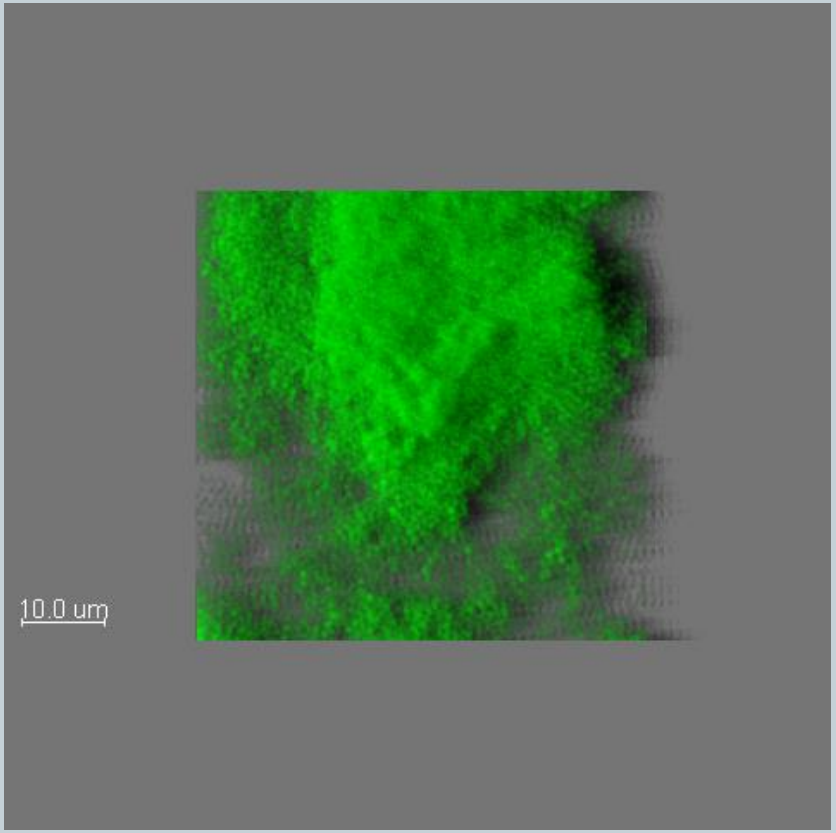
Discussion

- Strong approach to combining techniques to reveal structure and function for mixed biofilm
- The combination of GFP expressions with in situ hybridization with fluorescence-labeled 16S rRNA targeting probes
- Furthermore with FACS and cell sorting
- Each method has it's weakness

Results

- FISH





Further studies

- Do the experiment again:)
- Label the Acinetobacter also
- Change innoculation time of the two strains
- Detect gene expression together with hybridization

Our experiment

GROUP 2

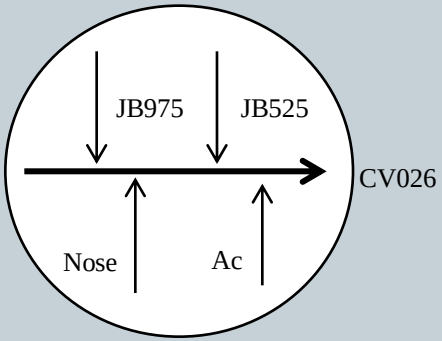
Our experiment

- Aim: To examine our own strains
- Isolates: *Acinetobacter* and a nose isolate

Methods

- BIOLOG

- Quorom sensing assay

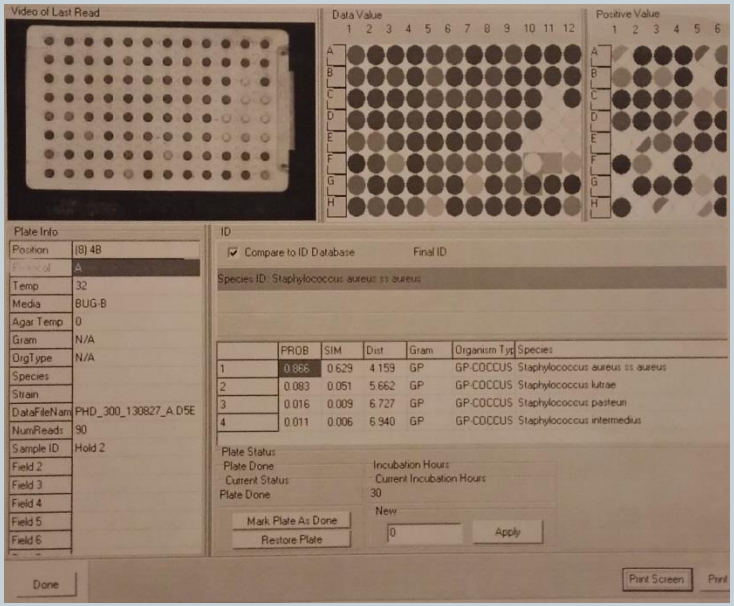


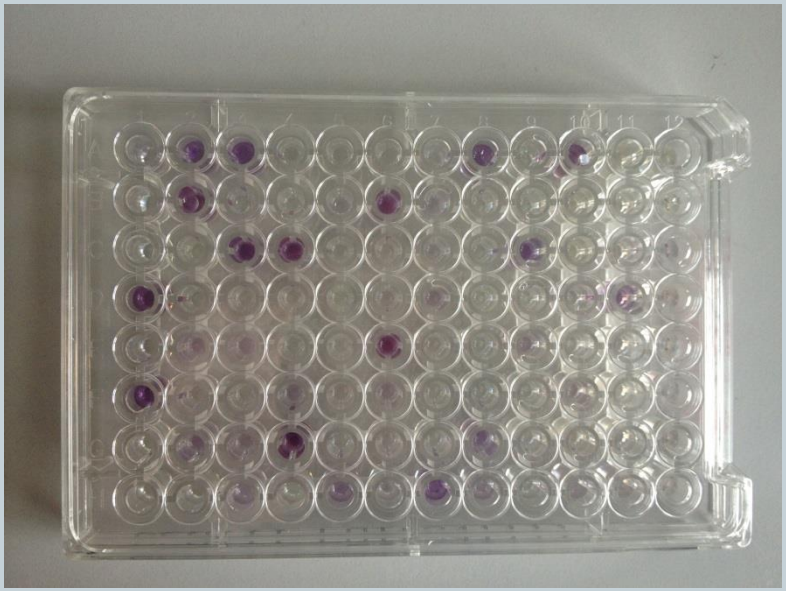
- Biofilm/flow cell experiment (stained with Cyto 9)

Results

- BIOLOG (only nose isolate)

Identified to be *Staphylococcus aureus* (Prob 0,866)



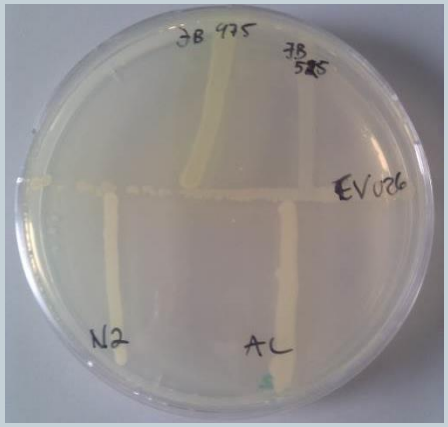


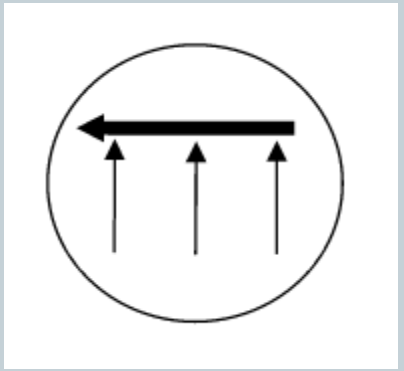
Results

- Quorum sensing assay

No results.

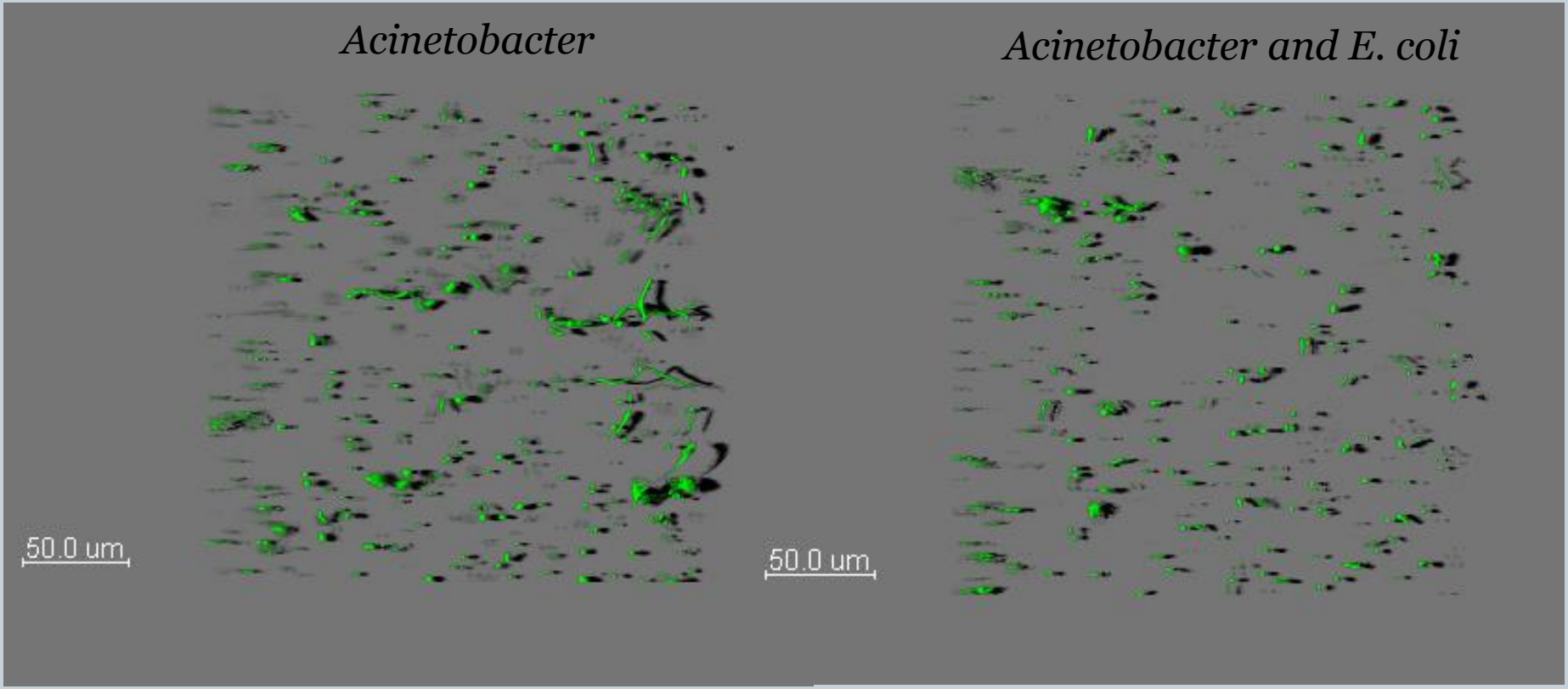
Problems with monitor strain CV026





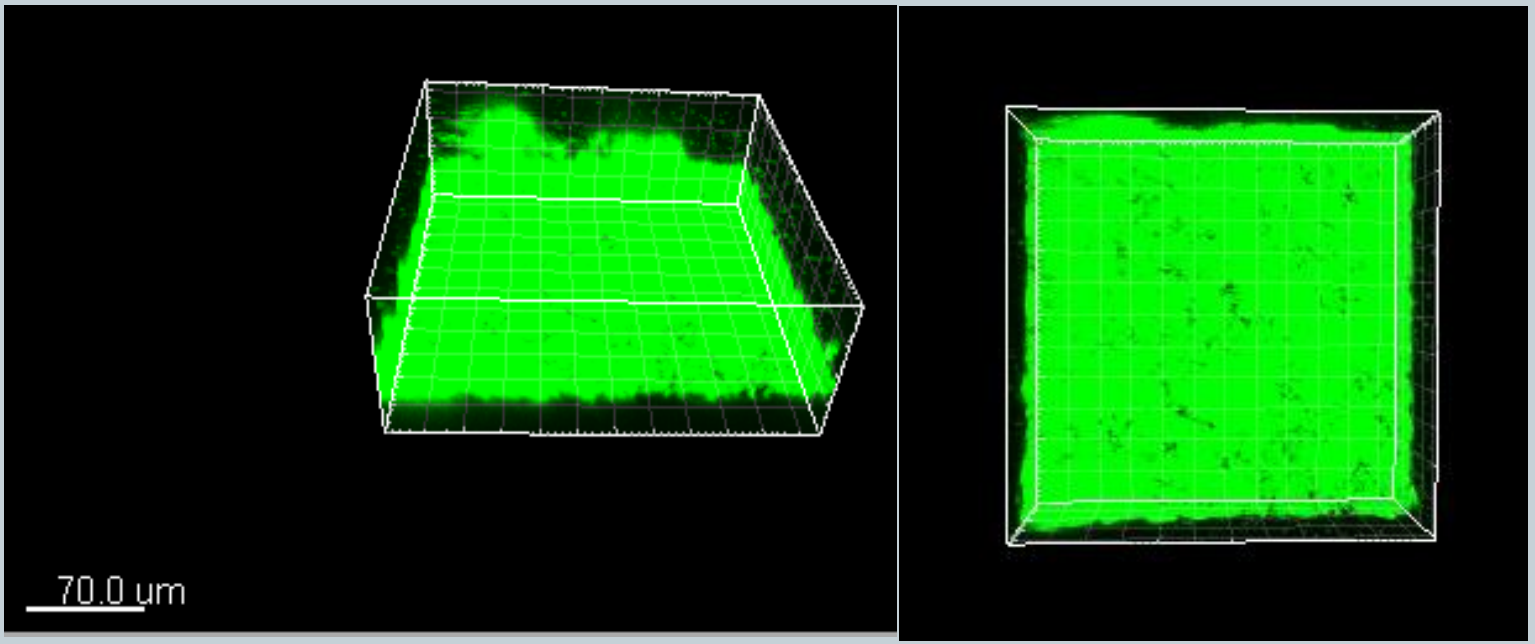
Results

- Biofilm/flowcells



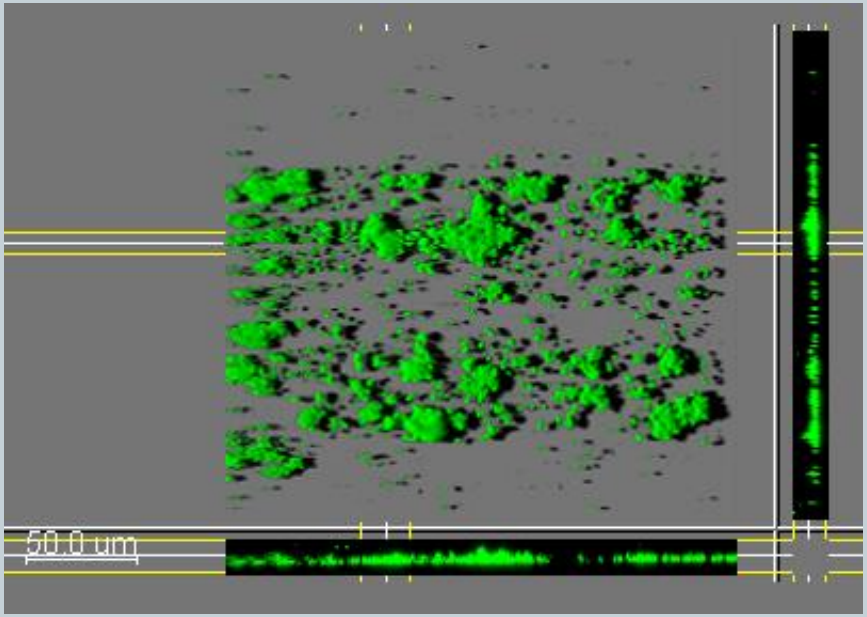
Results

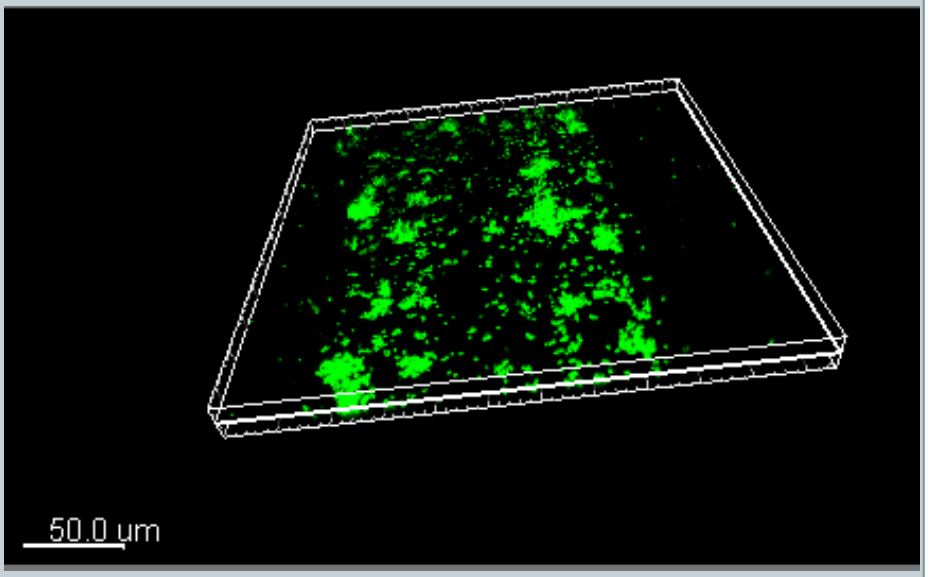
- *Acinetobacter*



Results

- *Staphylococcus aureus*





Discussion

- BIOLOG: Quick, knowlegde, many at one time
- Quorom sensing: again with a different monitor strain
- Biofilm/flow system

The end

Thank you for listening.